

Bohach and Schlievert, Infect Immun 57:2249 (1989)]. All of these toxins contain the residues underlined above, including the first 2 lysine residues (KK) and the QELD [Swaminathan *et al.* (1992) *ibid.*; Bohach and Schlievert (1987) *ibid.*; Couch *et al.* (1988) *ibid.*; Bohach and Schlievert (1989) *ibid.*]. The superantigen, pep M5 protein, also contains a region with limited homology (<50%) to pSEB(150-161) located near its C-terminus [Wang *et al.*, J Immunol 151:1419 (1993)].

REMARKS

This amendment is in response to the Office Action dated March 20, 2001. Based on discussions with the Examiner during an interview on June 28, 2001, Applicants filed a Continued Prosecution Application on August 20, 2001 with a request to suspend action which was granted on August 31, 2001 making the due date for response November 20, 2001.

Reconsideration of this application in view of the technical amendments and remarks made herein is respectfully requested.

Claims 50-92 are pending. Applicants have made technical amendments to claims 50, 51, 66-76, and 80 and to the specification. New claims 84-92 have been added. Support for amended claims 50, 51, 66-76 and 80 and new claims 84-92 can be found throughout the specification and the state of the art at the time the application to which priority is claimed was filed. The specification has been amended to correct typographical errors. No new matter has been added by the amendments to the specification or by the new and amended claims.

In accordance with 37 C.F.R. §1.121, applicants have provided (1) accurate instructions to amend the claims, (2) replacement claims in clean form herein, and (3) another version of the amended claims marked up to show all the changes relative to the previous version of the claims, which appears on an attached page.

## I. THE INVENTION

Applicants have discovered that certain isolated peptides, derived from pyrogenic exotoxins that induce toxic shock, including, but not limited to exotoxins B (SEB), SEA, TSST-1 of *Staphylococcus aureus* and SPEA of *Streptococcus pyogenes* are capable of eliciting a protective immune response against toxic shock, as well as directly antagonize toxin-mediated lymphocyte activation. These peptides are homologous to an amino acid sequence of a domain of such exotoxins which forms a central turn in the toxin molecule starting within  $\beta$ -strand 7 and connecting the  $\beta$ -strand 7, via short  $\alpha$ -strand 8, to  $\alpha$ -helix 4, and ending within  $\alpha$ -helix 4 (see Fig. 2) wherein said peptides do not have toxin agonist activity and are capable of antagonizing toxin-mediated activation of T lymphocytes. In SEB (SEQ ID NO:12), this domain encompasses amino acids 150-161 (SEQ. ID NO: 1). Such isolated peptides directly inhibit pyrogenic toxin-mediated induction of IL-2, INF-( and/or TNF- $\beta$  gene expression in normal peripheral blood mononuclear cells (PMBC). The expression of these genes is exquisitely sensitive to toxin (e.g., SEB) mediated activation. Likewise, antibodies against the peptides of the invention inhibit the expression of these genes, induced by exotoxins such as, but not limited to, SEB, a related toxin, SEA, a more distantly related toxin TSST-1, and SPEA, all of which can induce toxic shock in an individual.

The present application and a review of the prior art at the time of the application from which this application claims priority, clearly shows that this domain of the SEB not only manifests three-dimensional protein structural homology to the corresponding domain in other exotoxins, but also manifests amino acid sequence similarity to the corresponding domain in other exotoxins. The present invention, for the first time, shows that peptides homologous to this

domain, whose structure was known from the elucidation of the structures of several full length exotoxins at the time of filing (*see, e.g.* Roussel *et al.* *Nat. Struct. Biol.* 4:635-643 (1997); Schad *et al.* *EMBO* 14:3292-3301 (1995); Swaminathan *et al.* *Nature* 359:801-806 (1992); Jardetzky *et al.* *Nature* 368:711-718 (1994); Fields *et al.* *Nature* 384:188-192 (1996); and Prasad *et al.* *Biochemistry* 32:13761-13766 (1993)), are capable of antagonizing toxin-mediated activation of T lymphocytes and protect against lethal toxic shock (see Figs. 18 and 19). The inventors have additionally shown that such peptides that are homologous to this domain elicit lasting protective immunity to toxic shock. This is clearly a discovery of great magnitude. The overall similarity between the amino acid sequence of this domain in SEB and of the corresponding domains in other exotoxins may be as low as ~30%. **Of significant importance** is, for example, this domain in TSST-1 shares only 33% sequence similarity with the corresponding SEB domain, yet the domain folding is conserved. Nonetheless, the peptides of the invention (that share a higher homology with SEB) were capable of antagonizing toxin-mediated activation of T lymphocytes and of protecting against lethal shock induced by TSST-1.

The fact that a three-dimensional structural domain of a protein could provide a basis for functional activity is of great importance. Applicants wish to point out that it is well known in the art that three-dimensional structural domains often relate to a particular activity of a protein. This is discussed in detail, *e.g.* in Molecular Biology of the Gene, Watson, Hopkins, Roberts, Steitz and Weiner, Fourth Edition, The Benjamin/Cummings Publishing Company, Inc. (1987) (pp. 144-146; 162, attached hereto as Exhibit 1), where a domain is described as: "*a compact, folded part of the structure, that appears separate from the rest, as if it would be stable in solution on its own (which is often demonstrated to be the case)*". The structure-function relatedness is a most important factor.

To give a specific example, DNA binding and tetramerization domains of p53 were defined by proteolytic digestion of p53. See, Pavletich et al., 1993, *Genes Dev.* 7:2556-2464 (abstract attached hereto as Exhibit 2). Furthermore, Pavletich confirmed that these were structural domains by determining the three-dimensional structure of the domains. The x-ray crystallographic three-dimensional structure of the DNA binding domain elucidated how the domain bound to DNA. See Cho et al., 1994, *Science* 265:346-355 (abstract attached hereto as Exhibit 3). In addition, the three-dimensional structure of the tetramerization domain showed that in fact, this domain is in the form of a tetramer (tetramerization is known to be required for activity). See Jeffrey et al., 1995, *Science* 267:1498-1502 (abstract attached hereto as Exhibit 4). It is also well known that small peptides can have secondary structure. For example, the structure of MDM2 oncoprotein bound to a 15-amino acid transactivation domain peptide of p53 shows that the p53 peptide binds as an  $\alpha$ -helix. See Kussie et al., *Science* 8:948-953 (1996) (attached hereto as Exhibit 5). Therefore, Applicants wish to assert that defining a functional peptide by referring to its homology to a defined structural domain that is part of the overall structure of a full length protein does provide a detailed description of the peptide.

## II. REJECTIONS UNDER 35 USC §112 ¶2:

Claims 50-64 and 76-83 (the Examiner indicates that there were previously 84 claims but Applicants believe there were 83 and are now 92 by this Amendment) stand rejected under 35 USC §112, ¶ 2 as being vague and indefinite for using the term "substantially homologous." Applicants believe that this term is supported in the specification of the present invention and is well understood in the art.

For example, page 25, lines 21-27; page 26, lines 1-10 discusses sequence homologies among related pyrogenic toxins in the domain of such toxins that forms the "central

"turn" of such molecules, corresponding to amino acids 150-161 of SEB. The specification clearly provides that the peptides have KK and QELD motifs that are common to SEB, as well as to the related toxins SEA, SEC1, SEC2 and *S. pyogenes* exotoxin A (SPE A). Likewise, the meaning of homology can be ascertained by the disclosure that the related exotoxin proteins may share between 9/12-10/12 amino acid residues with SEB in the region of amino acids 150-161 of SEB (i.e., in the "central turn" domain of SEB and related exotoxins). *See, e.g.*, Example 2, page 36, lines 16-21, Example 6, page 41, lines 1-13. Moreover, any peptide within the scope of Claim 50 or 51, and claims dependent thereon, must also meet the functional limits set forth in the claim, which are also clear and understandable to someone of skill in the art, *i.e.* the isolated peptide does not have toxin agonist activity and is capable of antagonizing toxin-mediated activation of T lymphocytes.

As indicated above, it is well known that particular functions of proteins can be linked to defined three-dimensional domains within the proteins and that domains may have the particular function even in the absence of the rest of the protein (*see* discussion of p53 above and Exhibit 1). For this reason, Applicants assert that defining a peptide as having an amino acid sequence that is homologous to an amino acid sequence of a defined domain of a protein and further functionally defining the peptide does definitively describe the peptide.

Applicants have amended the claims to indicate that the peptides of the present invention have an amino acid sequence homologous to an amino acid sequence of a domain of a pyrogenic exotoxin wherein said peptides do not have toxin agonist activity and are capable of antagonizing toxin-mediated activation of T lymphocytes. Further definition is now given to the meaning of "homologous to" indicating that it requires that the peptides do not have toxin agonist activity and are capable of antagonizing toxin-mediated activation of T lymphocytes

making this term definite for the purposes of Section 112. In view of these amendments, Applicants respectfully request that the Section 112 rejection be withdrawn.

The Examiner also rejected claims 50-84 (83) in the previous Office Action dated October 2, 2000 under 35 U.S.C. § 112, ¶ 2 as being vague and indefinite for using the term derivatives. In the Office Action dated March 20, 2001, the Examiner withdrew this rejection based on Applicants arguments in Applicants Response dated January 2, 2001. However, during the Interview with the Examiner on June 28, 2001, the Examiner indicated that this rejection would be reinstated in future Office Actions. Therefore, Applicants have removed the phrase "and derivatives thereof" from the claims and respectfully request that this rejection be permanently withdrawn. Applicants however wish to assert that the present application does clearly teach many derivatives and maintain the arguments presented in their previous response.

### III. REJECTIONS UNDER 35 USC § 102(b)

Claims 50-84 (83) stand rejected under 35 USC § 102(b) as allegedly anticipated by Tseng et al., Infect. Immun. 63(8): 2880-85 (1995) ("Tseng et al.").

Amended claim 50 is directed to an isolated and purified peptide homologous to an amino acid sequence of a domain of the pyrogenic toxins that forms a central turn in the molecule (See Fig. 2 and Example 2) wherein said isolated peptide does not have toxin agonist activity and is capable of antagonizing toxin-mediated activation of T lymphocytes. Support for this amendment can be found throughout the specification and particularly Figure 3, Example 3, and page 18, lines 3-6. Claim 52 is directed to a peptide according to claim 50 having the amino acid sequence as set forth in SEQ ID NO: 1. Claims 52-65 define various derivatives of the isolated peptides (e.g., dimerized and multimerized forms, conformationally stabilized forms and peptides having N- and C-terminal additions) and specific sequences thereof, while the

remaining claims define compositions comprising the isolated peptides. Thus, the present invention is directed to isolated peptides that are homologous to, but do not constitute a full length toxin protein because they do not have toxin agonist activity. In addition, the peptides of the present invention are capable of antagonizing toxin-mediated activation of T lymphocytes.

On the other hand, Tseng et al. teach administration of a SEB toxoid (a full length protein, not a peptide) in microspheres to monkeys in order to elicit neutralizing antibodies to SEB (toxin). Those monkeys that produced such antibodies to the toxoid appeared to survive a subsequent aerosol challenge with SEB. But, Tseng et al. neither teach nor suggest that the toxoid itself antagonizes SEB activity.

It is well known that a toxoid is a chemically-modified full length toxin protein that retains the antigenicity (immunogenicity) of a toxin protein. Thus, immunization with a toxoid can lead to production of an immune response to a toxin that, in some circumstances, is protective. For example, this is the basis for immunization against diphtheria and tetanus.

In Tseng et al., SEB toxin (full length protein) was treated with formalin and then alum precipitated to produce the toxoid used for immunization. However, Tseng et al. never isolated any peptides corresponding to a particular domain of SEB as in the present invention, nor tested the ability of such isolated peptides themselves to inhibit SEB and other toxin (e.g., SEA, TSST-1) -mediated activities, such as activation of IL-2, IFN-( and TNF- $\beta$  gene expression, nor produced antibodies to the specific peptide that also inhibit toxin-mediated T cell activation, as in the present invention.

Moreover, as documented in the present specification (*see, e.g.,* page 5, lines 4-10; page 31, lines 1-5; page 43, lines through page 45, line 19 and page 51, lines 10-16), the ability to elicit antibodies to SEB by administration of toxoid, as shown in Tseng et al., is not

predictive of whether such antibodies will antagonize (i.e., inhibit) toxin-mediated T-cell activation, nor inhibit toxin-mediated gene expression, as in the present invention nor is it predictive of whether such a toxoid does not have toxin agonist activity. In fact, in some cases, antibodies to certain portions or domains of SEB actually potentiated the toxin activity of different toxins (*see* Figs. 13 [SEB] and 14 [SEA]).

Since the isolated peptides of the present invention do not have toxin agonist activity but are capable of antagonizing toxin-mediated activation of T lymphocytes, they cannot read on a full length protein, and specifically on the toxoid of Tseng et al. which comprises full length SEB, because by definition the full length protein is capable of toxin agonist activity nor can they be anticipated by Tseng et al.

For a reference to anticipate a claim under 35 USC § 102(b), the reference must teach each and every limitation of the claim. Scripps Clinic & Research Fdn. v. Genentech, Inc., 18 U.S.P.Q. 2d 1001, 1010-1011 (Fed. Cir. 1991).

Tseng et al. clearly does not teach each and every element of the invention as claimed, which is required for a rejection under Section 102, thereby obviating this rejection. Moreover, Tseng et al. does not even suggest the presently claimed invention. There is clearly no suggestion whatsoever in Tseng et al. of the claimed isolated peptides and compositions comprising them. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

#### IV. REJECTION UNDER 35 U.S.C. § 102(e):

The Examiner has further rejected claims 50-84 under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 5,705,151 of Dow et al. ("Dow et al."). The Examiner contends that Dow et al. teach a peptide (Dow et al. SEQ ID NO:2) which is 99.8% identical to the

claimed amino acid sequence of SEQ ID NO:12 and which is capable of antagonizing toxin-mediated activation of T lymphocytes. In addition, the Examiner contends that Dow et al. teach a peptide (Dow et al. SEQ ID NO:2) that is substantially homologous to any one of peptides of SEQ ID NO:1-12. Applicants maintain that Dow et al. do not anticipate the claims of the present invention.

Dow et al. only discloses full-length proteins and does not teach that a portion of that full length protein can be capable of antagonizing toxin mediated activation of T lymphocytes. Moreover, Dow et al. do not teach peptides that do not have toxin agonist activity. The skilled artisan could not look to Dow et al. to design the peptides of the present invention which do not have toxin agonist activity but retain the ability to antagonize toxin-mediated activation of T lymphocytes. Since the claims as amended are directed to peptides homologous to an amino acid sequence of a pyrogenic exotoxin which do not have toxin agonist activity but are capable of antagonizing toxin-mediated activation of T lymphocytes, Applicants assert that Dow et al. does not anticipate the present invention and respectfully submit that the rejection under 35 U.S.C. § 102(e) be withdrawn.

V. NEW CLAIMS:

Applicants have added new claims 84 through 92. Support for new claims 84-86 is found throughout the specification. Claims 87-92 are directed to peptides having a consensus amino acid sequence and specifically peptides which do not have toxin agonist activity having the following sequences:

(1) KXaa<sub>(3)</sub>TXaaQEXaaD (claim 87)

(2) KKXaa<sub>(6)</sub>LD (claim 88)

- (3) charged amino acid-(X)2-hydrophobic amino acid-X-hydrophobic amino acid-polar amino acid-polar amino acid-hydrophobic amino acid-D (claim 89).
- (4) Xaa<sub>(2)</sub>KXaa<sub>(3)</sub>TXaaQEXaaD (claim 90)
- (5) Xaa<sub>(2)</sub>KKXaa<sub>(6)</sub>LD (claim 91)
- (6) Xaa<sub>(2)</sub>-charged amino acid-(X)2-hydrophobic amino acid-X-hydrophobic amino acid-polar amino acid-polar amino acid-hydrophobic amino acid-D (claim 92).

Support for the consensus amino acid sequences of claim 87-92 is found in the peptides of the present invention (SEQ ID NOs:1-11). In addition, as indicated in the specification and above, the claims are further supported by full length exotoxin sequences known in the art at the time the application from which the present application claims priority was filed. The specification clearly provides that the peptides have KK and QELD motifs that are common to SEB, as well as to the related toxins SEA, SEC1, SEC2 and *S. pyogenes* exotoxin A (SPE A). In addition, attached as Exhibit 6, please find a list of sequences which were known at the time of filing of the application to which the present application claims priority and which further support the consensus sequences claimed in claims 87-92.

VI. CONCLUSION

In view of the amendments to the claims and the remarks herein, Applicants maintain that Claims 50-92 are now in condition for allowance. A Notice of Allowance is earnestly solicited.

Applicants believe that no fee is required in connection with this communication. However, if a fee is required, the Commissioner is hereby authorized to charge the fee to Deposit Account 02-4377. A duplicate of this sheet is enclosed.

Respectfully submitted,



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Attachments